Precise Correction of Disease Mutations in Induced Pluripotent Stem Cells Derived From Patients With Limb Girdle Muscular Dystrophy.

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Public Summary:
This study demonstrates how it is possible to correct precisely the mutations that exist in patients with genetic diseases. In the work, we focused on two specific patients who have different forms of limb girdle muscular dystrophy. For each patient, we used the CRISPR/Cas9 genome editing method to change the mutated base in the patient's DNA to the correct base. CRISPR/Cas9 is a recently-developed genetic engineering method that allows a researcher to make precise changes in the DNA of the chromosomes. For one of the patients, we also applied two other methods, called DICE and THRIP, to add a healthy version of the mutated gene to a safe location in the patient's chromosomes. These alternative strategies are faster and more efficient than the CRISPR/Cas9 method. In all cases, we showed that the corrected cells were now able to make the proteins that were missing or inactive previously in the patient cells. By changing the DNA sequence in stem cells derived from the patients, the resulting corrected stem cells could potentially be used in cell therapy strategies to improve the clinical condition of these patients.

Scientific Abstract:
Limb girdle muscular dystrophies types 2B (LGMD2B) and 2D (LGMD2D) are degenerative muscle diseases caused by mutations in the dysferlin and alpha-sarcoglycan genes, respectively. Using patient-derived induced pluripotent stem cells (iPSC), we corrected the dysferlin nonsense mutation c.5713C>T; p.R1905X and the most common alpha-sarcoglycan mutation, missense c.229C>T; p.R77C, by single-stranded oligonucleotide-mediated gene editing, using the CRISPR/Cas9 gene-editing system to enhance the frequency of homology-directed repair. We demonstrated seamless, allele-specific correction at efficiencies of 0.7-1.5%. As an alternative, we also carried out precise gene addition strategies for correction of the LGMD2B iPSC by integration of wild-type dysferlin cDNA into the H11 safe harbor locus on chromosome 22, using dual integrase cassette exchange (DICE) or TALEN-assisted homologous recombination for insertion precise (THRIP). These methods employed TALENs and homologous recombination, and DICE also utilized site-specific recombinases. With DICE and THRIP, we obtained targeting efficiencies after selection of ~20%. We purified iPSC corrected by all methods and verified rescue of appropriate levels of dysferlin and alpha-sarcoglycan protein expression and correct localization, as shown by immunoblot and immunocytochemistry. In summary, we demonstrate for the first time precise correction of LGMD iPSC and validation of expression, opening the possibility of cell therapy utilizing these corrected iPSC.Molecular Therapy (2016); doi:10.1038/mt.2016.40.